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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
09/761,893	01/17/2001	Shih-Chieh Hung	11709-003001	6011

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EXAMINER	
DUNSTON, JENNIFER ANN	
ART UNIT	PAPER NUMBER
1636	

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Please find below and/or attached an Office communication concerning this application or proceeding.

The time period for reply, if any, is set in the attached communication.

Office Action Summary	Application No.	Applicant(s)	
	09/761,893	HUNG ET AL.	
	Examiner	Art Unit	
	Jennifer Dunston	1636	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE *3* MONTH(S) OR THIRTY (30) DAYS, WHICHEVER IS LONGER, FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *22 September 2010 and 09 November 2010*.

2a)☐ This action is **FINAL**. 2b)☒ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *1,4,6,9-20,34-38 and 41* is/are pending in the application.

4a) Of the above claim(s) *12-20* is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *1,4,6,9-11,34-38 and 41* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☒ The drawing(s) filed on *17 January 2001* is/are: a)☒ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

11)☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

12)☒ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☒ All b)☐ Some * c)☐ None of:

1.☒ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☐ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☐ Information Disclosure Statement(s) (PTO/SB/08) Paper No(s)/Mail Date _____.
4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____
5) ☐ Notice of Informal Patent Application
6) ☐ Other: _____.

DETAILED ACTION

Continued Examination Under 37 CFR 1.114

A request for continued examination under 37 CFR 1.114, including the fee set forth in 37 CFR 1.17(e), was filed in this application after final rejection. Since this application is eligible for continued examination under 37 CFR 1.114, and the fee set forth in 37 CFR 1.17(e) has been timely paid, the finality of the previous Office action has been withdrawn pursuant to 37 CFR 1.114. Applicant's submission filed on 9/22/2010 has been entered.

Receipt is acknowledged of an amendment, filed 9/22/2010, in which claim 1 was amended, and claim 41 was newly added. Claims 1, 4, 6, 9-20, 34-38 and 41 are pending.

Election/Restrictions

Applicant elected Group I without traverse in the reply filed on 9/4/2001.

Claims 12-20 are withdrawn from further consideration pursuant to 37 CFR 1.142(b) as being drawn to a nonelected invention, there being no allowable generic or linking claim. Election was made **without** traverse in the reply filed on 9/4/2001.

Currently, claims 1, 4, 6, 9-11, 34-38 and 41 are under consideration.

Claim Objections

Claim 1 is objected to because of the following informalities:

1. The comma after the word "plate" in line 6 should be deleted to improve the grammar of the claim.

2. The phrase "and pores are" in line 7 should be replaced with the phrase "with pores of" to improve the grammar of the claim.

3. The word "and" in line 13 should be deleted to improve the grammar of the claim.

4. The period at the end of line 14 must be deleted. The period should be replaced with a semicolon followed by the word "and" to improve the grammar of the claim.

Claims 4, 6, 9-11, 34-38 and 41 depend from claim 1 and are objected to for the same reasons applied to claim 1.

Appropriate correction is required.

Claim 34 is objected to because of the following informalities: the phrase "said the mesenchymal stem cell adhering material" should be replaced with the phrase "said mesenchymal stem cell adhering material" or "the mesenchymal stem cell adhering material." Use of "said" and "the" together is unnecessary. Appropriate correction is required.

Claim 35 is objected to because of the following informalities: the phrase "said the mesenchymal stem cells" should be replaced with the phrase "said mesenchymal stem cells" or "the mesenchymal stem cells." Use of "said" and "the" together is unnecessary. Claims 36-38 depend from claim 35 and are objected to for the same reason applied to claim 35. Appropriate correction is required.

Claim Rejections - 35 USC § 112

The following is a quotation of the second paragraph of 35 U.S.C. 112:

> The specification shall conclude with one or more claims particularly pointing out and distinctly claiming the subject matter which the applicant regards as his invention.

Claims 1, 4, 6, 9-11, 34-38 and 41 are rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention. This is a new rejection.

Claim 1 recites the limitation "the mesenchymal stem cell adhering material" in lines 6-7. There is insufficient antecedent basis for this limitation in the claim. It would be remedial to amend the phrase to recite "a mesenchymal stem cell adhering material."

Claim 1 recites the limitation "the other small-sized hematopoietic and non-hematopoietic cells" in line 9. There is insufficient antecedent basis for this limitation in the claim. It would be remedial to amend the phrase to delete the phrase "the other" so it recites "small-sized hematopoietic and non-hematopoietic cells."

Claims 4, 6, 9-11, 34-38 and 41 depend from claim 1 and are rejected for the same reasons applied to claim 1.

The following is a quotation of the first paragraph of 35 U.S.C. 112:

> The specification shall contain a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the same and shall set forth the best mode contemplated by the inventor of carrying out his invention.

Claim 41 is rejected under 35 U.S.C. 112, first paragraph, as failing to comply with the written description requirement. The claim(s) contains subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that

the inventor(s), at the time the application was filed, had possession of the claimed invention.

This is a new matter rejection.

In the reply filed 9/22/2010, new claim 41 was added. The claim limits the pores of the upper plate used in the method of claim 1 to about 0.4 to 20 microns in diameter.

The reply filed 9/22/2010 indicates that the new range is within the scope of the originally filed range of 0.4 to 40 microns and also asserts that the range is not new matter based upon the cited case law.

The originally filed claims and specification teach the method where the pores of the upper plate are from about 0.4 to 40 microns in diameter (e.g., specification, page 7, paragraph bridging pages 8-9). The specification does not specifically disclose the range of 0.4 to 20 microns and does not exemplify or specifically teach the use of pores of 20 microns. Thus, the subgenus range now claimed is not supported by the generic disclosure of the originally filed specification.

In the decision in In re Wertheim, 541 F.2d 257, 191 USPQ 90 (CCPA 1976), the ranges described in the original specification included a range of "25%- 60%" and specific examples of "36%" and "50%." A corresponding new claim limitation to "at least 35%" did not meet the description requirement because the phrase "at least" had no upper limit and caused the claim to read literally on embodiments outside the "25% to 60%" range, however a limitation to "between 35% and 60%" did meet the description requirement. See MPEP 2163.05. In the instant case, the specification does not provide any specific examples within the broader range of 0.4 to 40 microns. Thus, the range of 0.4 to 20 microns does not meet the description requirement.

The decision in Waner-Jenkinson Co., Inc. v. Hilton Davis Chemical Co., 41 USPQ2d 1865 (U.S. 1997) is not related to new matter. Each case must be evaluated on its own merits. The issue of "new matter" would not be raised when the originally filed specification provides support a later claimed range. For example, in the instant application, if an original claim to the method where any pore size is used was later amended to limit the pore size to 0.4 to 40 microns, this would not be new matter. The originally filed specification provides support for about 0.4 to 40 microns. However, the originally filed disclosure does not provide support for a pore size of 20 microns or the range of 0.4 to 20 microns. Thus, claim 41 constitutes new matter.

The original specification, drawings and claims were thoroughly reviewed and no support could be found for the amendment. Accordingly, the amendment is a departure from the specification and claims as originally filed.

Claims 1, 4, 6, 9-11, 34-38 and 41 are rejected under 35 U.S.C. 112, first paragraph, as failing to comply with the enablement requirement. The claim(s) contains subject matter which was not described in the specification in such a way as to enable one skilled in the art to which it pertains, or with which it is most nearly connected, to make and/or use the invention. This rejection was made in the Office action mailed 8/13/2010 and has been rewritten to address the amendments to the claims in the reply filed 9/22/2010.

Enablement is considered in view of the Wands factors (MPEP 2164.01(A)). These include: nature of the invention, breadth of the claims, guidance of the specification, the existence of working examples, state of the art, predictability of the art and the amount of

experimentation necessary. All of the Wands factors have been considered with regard to the instant claims, with the most relevant factors discussed below.

Nature of the invention: The claims are drawn to a method for isolating mesenchymal stem cells (MSCs) from bone marrow aspirate, and the claims explicitly require the separation of MSCs from small hematopoietic stem cells (HSCs) and other non-hematopoietic cells.

Independent claim 1 comprises the following method steps: (a) providing a cell mixture comprising MSCs and other cells; (b) seeding and culturing the cell mixture in a culture device comprising an upper plate with pores and a lower plate base, said upper plate made of MSC adhering material and has pores of about 0.4 to 40 microns in diameter, where MSCs adhere and are cultured, and the lower plate base, where the other small-sized HSCs adhere following passing through the pores in the upper plate, said culturing with medium containing factors that stimulate MSC growth without differentiation and allows for the selective adherence of only MSCs to the upper plate surface; (c) removing non-adherent cells on the upper plate by changing medium; and (d) collecting mesenchymal stem cells from the upper plate. Dependent claims 4, 6, 9 and 10 further limit the characteristics of the MSC. Claims 11 and 35-38 further limit the culturing conditions. Claim 11 is drawn to the use of culture medium comprising 10% fetal bovine serum-supplemented Dulbecco's modified Eagle's medium containing 1g/L of glucose. Dependent claim 34 limits the mesenchymal stem cell adhering material to plastic. Dependent claim 41 limits the pore size to about 0.4 to 20 microns in diameter.

The claimed invention seeks to exploit the biological and physical characteristics of MSCs to provide an isolated population of MSCs. With regard to the biological properties, the claim requires the selective adherence of MSCs to the top plate. With regard to physical

properties, the claim requires size selection of MSCs. Specifically, the upper plate with pores must separate MSCs from HSCs. The upper plate must retain the MSCs while allowing HSCs to pass through the pores.

Breadth of the claims: The claims broadly encompass the use of a culture device comprising an upper plate that contains pores of about 0.4 to 40 microns. The complex nature of the subject matter of this invention is greatly exacerbated by the breadth of the claims.

Guidance of the specification and existence of working examples: The specification envisions using the physical and biological characteristics of MSCs to isolate MSCs from a cell mixture. Specifically, the specification envisions using a difference in cell size and a difference in adherence to isolate MSCs (e.g., page 7, lines 10-17). The specification envisions using a plate with pores, where the pore size is defined functionally as being "sufficient for separating mesenchymal stem cells from other cells (e.g. haematopoietic stem cells)" (page 7, lines 24-27). The specification asserts that a preferable pore size is from about 0.4 to 40 microns in diameter (e.g., page 7, lines 27-29; page 8, lines 29-30).

A working example of the claimed method is presented on page 11-12 of the specification; however, the working example **does not disclose the pore size** of the upper plate. Percoll fractionated or un-fractioned bone marrow cells in DMEM-LG with 10% FBS and antibiotics were seeded into a culture device at a density of $10^6/cm^2$ (page 11, lines 20-25). The design specifics of the culture device used in the working example are not disclosed. The specification notes that HSCs and non-adherent cells were removed with changes in medium (page 12, lines 4-6; page 14, lines 18-20). The cells retained by the upper plate were analyzed and found to have characteristics consistent with a MSC phenotype (e.g., page 12, line 16 to page

15, line 28). At page 14, the specification discusses the characteristics of the cells that are collected on the bottom plate (i.e., the cells that pass through the undefined pores). The cells were described as having a small size, polygonal shape, and little renewal capacity (page 14, lines 7-9). The specification states that the cells "were believed to be haematopoietic cells" (page 14, line 10).

The specification does not provide any characterization of the cells that are collected on the lower plate and are "believed to be haematopoietic cells." The cell surface markers of the cells were not analyzed (e.g., CD34). The ability of the cells to differentiate along the hematopoietic lineage or perform hematopoietic reconstitution is not disclosed.

Predictability and state of the art: The state of the art with regard to the separation of MSCs and HSCs based upon size selection through pores was underdeveloped at the time the invention was made. Prockop et al (US Patent No. 7,374,937 B1, effective date March 14, 2000, cited in a prior action) teaches the isolation of MSCs from bone marrow aspirates obtained from the iliac crest of normal human donors (e.g., column 24, line 24 to column 35, line 5). The non-adherent cells were removed, and the adherent cells were harvested (e.g., paragraph bridging columns 34-35). Prockop et al assayed the MSCs for size and granularity by forward light and side light scattering by FACS (e.g., column 36, lines 9-41). When the MSCs were plated at a low density, a population of large cells with a medium content of granules was observed (mature MSCs or mMSCs) along with two populations of small cells: (1) small and agranular cells referred to as recycling stem cells-1 (RS-1); and (2) small and granular cells referred to as recycling stem cells-2 (RS-2) (e.g., column 36, lines 31-34). Prockop et al teach that a polycarbonate membrane with a pore size of 10 micrometers separates the mMSCs from the RS

cells (e.g., column 39, line 60 to column 40, line 42). Prockop et al teach that the small cells that pass through the pores are CD34-negative (e.g., Figure 26). The present specification teaches that HSCs obtained from bone marrow are CD34-positive (e.g., page 14, lines 26-27). Thus, the small cells separated from the MSC of bone marrow aspirate using a 10 micron filter do not appear to be HSCs. Further evidence that the small cells are not HSCs is provided by the post filing art. Colter et al teach that the RS cells are capable of adopting the same cell fates as MSCs in that they are capable of differentiating into osteoblasts, adipocytes and cartilage (Colter et al. Proceedings of the National Academy of Sciences, USA, Vol. 98, No. 14, pages 7841-7845, July 2001; e.g., paragraph bridging pages 7843-7844). Accordingly, it would be unpredictable to use a filter containing pores of 10 micrometers to separate MSCs from HSCs of bond marrow aspirate.

Applicant's own post-filing art teaches that MSCs were isolated from human bone marrow aspirates by the use of a "unique method that included a specially designed culture device, which was a plastic culture dish comprising a plate with 3-μm pores to sieve out MSCs from bone marrow aspirates" (Hung et al. Stem Cells, Vol. 20, pages 249-258, 2002; e.g., page 250, right column). The specific culture device used was a 10-cm plastic culture dish comprising a plate with 3-μm pores sold as a Transwell® device by Corning Inc. (e.g., page 251, paragraph bridging columns). The features of this device, such as the 3-μm pore size, are not taught by the present specification.

The post filing art teaches that the cells collected on the lower plate base have never been characterized (Zuba-Surma et al. Cytometry Part A, Vol. 75A, pages 4-13, 2009; e.g., page 11, right column, last full paragraph). Given the lack of characterization of the small cells, and the

evidence provided by Prockop et al and Colter et al that other small cells present in bone marrow are not HSCs, it would be unpredictable to separate MSCs from HSCs using a device with any size pores, or pores ranging from 0.4-40 microns.

Taichman et al (Blood, Vol. 89, No. 4, pages 1165-1172, February 1997) teach that $CD34^+$ hematopoietic bone marrow cells were seeded into the top chamber of Transwell® dual-chambered 24-well plates with a 0.4 μm pore size (e.g., page 1166, paragraph bridging columns). Taichman et al provide evidence that HSCs do not pass through 0.4 μm filters, because the cells can be cultured on the surface of such filters. Further evidence that cells do not pass through 0.4 μm filters is provided by US Patent Application Publication No. 2008/0085555 (e.g., paragraph [0045] teaches that pore sizes of 0.1 to 1 μm do not allow the passage of cells), US Patent Application Publication No. 2005/0265978 (e.g., paragraph [0161], 0.4 μm membrane prevents movement of cells), US Patent Application Publication No. 2010/0093077 (e.g., Figure 12, HSCs grow on 0.4 μm filters), and US Patent Application Publication No. 2005/0181381 (e.g., paragraph [0196], both MSCs and HSCs grow on 0.4 μm filters). Accordingly, it would be unpredictable to use a plate with pore sized of about 0.4 to 20 microns in diameter or 0.4 to 40 microns in diameter to separate MSCs from HSCs.

Amount of experimentation necessary: The quantity of experimentation needed to carry out the claimed invention is large. One would be required to determine the pore sizes that provide separation of MSCs from HSCs obtained from bone marrow aspirate. The prior art teaches that pores of 10 μm do not separate MSCs from HSCs; rather, pores of 10 μm separate large MSCs from small MSCs (RS cells). Furthermore, the specification does not teach the pore sized used in the working example. Without the guidance of the post filing art, it would require a

large amount of experimentation to determine the pore size that provides the result obtained in the specification. The post-fling art teaches a pore size of 3 μm was used, and the specification discloses any pore size, or preferably about 0.4 to 40 μm. Even if one were to use a pore size of 3 μm, there is no evidence on the record that the small cells obtained on the lower plate are HSCs. To determine if the cells are HSCs, one would be required to determine the cell surface markers and test the ability of the cells to differentiate along the hematopoietic lineage or perform hematopoietic reconstitution. The outcome of any experiment relying upon the guidance of the specification and prior art is unpredictable, and the type of experimentation required is not routine in the art.

In view of the breadth of the claims and the lack of guidance provided by the specification as well as the unpredictability of the art, the skilled artisan would have required an undue amount of experimentation to make and/or use the claimed invention. Therefore, claims 1, 4, 6, 9-11, 34-38 and 41 are not considered to be enabled by the instant specification.

Response to Arguments - 35 USC § 112

With respect to the rejection of claims 1, 4, 6, 9-11, 34-38 and 41 under 35 U.S.C. 112, first paragraph (enablement), Applicant's arguments filed 9/22/2010 have been fully considered but they are not persuasive.

The response asserts that it does not matter whether the small cells that pass through the pores are hematopoietic cells or not.

This argument is not found persuasive. The claims specifically require hematopoietic stem cells (HSCs) to pass through the pores of the upper plate and to adhere to the lower plate base. See step (b) of independent claim 1.

The response asserts that based on the teachings of the prior art, one can use an upper plate with a pore size of 0.4 to 40 microns to separate MSCs from other small size cells.

While one could separate MSCs from other small cells using pores of 0.4 to 40 microns, one could not apply this range of pore sizes to the separation of MSCs from HSCs as required by the claims. See step (b) of independent claim 1.

For these reasons, and the reasons made of record in the previous office actions, the rejection is maintained.

Conclusion

No claims are allowed.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Jennifer Dunston whose telephone number is (571)272-2916. The examiner can normally be reached on M-F, 9 am to 5 pm.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's acting supervisor, Joanne Hama can be reached on 571-272-2911. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free). If you would like assistance from a USPTO Customer Service Representative or access to the automated information system, call 800-786-9199 (IN USA OR CANADA) or 571-272-1000.

/Jennifer Dunston/
Primary Examiner
Art Unit 1636